



2.4.10 Calibration Standards for Microplate Readers and Spectrophotometers



Description

Various national and international regulations and standards, such as Good Laboratory Practice (GLP) or ISO 9001:2000, demand that the performance of spectrophotometers must be checked at regular intervals.

The set of secondary standards presented here enables the accuracy of spectrophotometers to be checked with respect to

- the ordinate (absorbance) in the visible range of the spectrum at the wavelengths 440 nm, 465 nm, 546 nm, 590 nm and 635 nm
- the abscissa (wavelength) in the ultraviolet and visible range at the wavelengths 279 nm, 361 nm, 454 nm, 536 nm and 638 nm.

The calibration set consists of three grey glass filters for checking the absorbance accuracy, and a holmium oxide glass filter for checking the wavelength accuracy. The glass filters are mounted in precision aluminium frames. They are designed for use with the standard 10 mm cell holder provided with spectrophotometers. The set, along with an empty mount, is supplied in a sturdy wooden case. For identification purposes, the set number is engraved on each filter mount. The absorbance values and/or peak position wavelengths of each filter are quoted in the accompanying calibration certificate. A copy of the values, for laboratory use, can be found on the inside face of the case lid.

Grey Glass Filters

The grey filters marked F2, F3, and F4 are NG 11, NG 5 and NG 4 glasses respectively, supplied by SCHOTT Glaswerke in Germany. These glasses have been selected because of their homogeneity and stability, and because of their generally uniform transmission values in the visible range of the spectrum. The thickness of the glass filters has been chosen to provide nominal absorbance values of 0.25 A, 0.5 A and 1 A.

Holmium Oxide Glass Filter

The slightly yellow filter marked F1 is made of a silica glass doped with holmium oxide (H₂O₃). The element holmium exhibits a number of sharp absorption peaks throughout the UV, visible, and NIR spectral ranges. Because the exact positions of the holmium peaks vary slightly for each production batch, the filters are calibrated individually.



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Didymium Glass Filter

As an alternative the didymium glass filter 666-F7 made of SCHOTT BG 36 can be supplied. It is used to check the wavelength accuracy in the visible spectral range and the absorbance values in the UV.

Microplate Reader Standard

The photometric accuracy microplate readers can be checked using the secondary standard **666.013**. The microplate reader has the same dimensions as a microplate and has windows with a 6.6 mm diameter at the same positions as the 96 wells. Grey glass stripes, with varying absorption values (see table on the following page), are located behind groups of 16 windows.

General Notes

The calibration of the secondary standards is accomplished through the use of a high performance UV/VIS/NIR spectrophotometer. This instrument is used exclusively for calibration purposes and is checked for its accuracy at fixed intervals of time. For calibration, a set of primary standards from the “National Institute of Standards and Technology” (NIST, U.S.A.) is used.

The calibration standards must be treated with special care if they are to retain their validity. Scratches, dirt, and corrosion on the optical surfaces can easily introduce substantial errors.

The calibration standards are to be considered measuring equipment in accordance with international standards and should be recalibrated at regular intervals (see DIN ISO 9901, section 7.6 Control of Inspection and Measuring Equipment).

We recommend that the standards are recalibrated at least every 24 months.

The filter or sets of filters which are sent to us to be recalibrated will be dismantled, cleaned and calibrated. They will then be returned to you with a new certificate. We reserve the right to replace damaged filters, especially ones showing marked deviations from the nominal values, at the customer’s expense.

Successful work with secondary standards is strongly influenced by the settings of the instrument. For this reason extensive handling instructions are provided with each set of filters. All filters can also be ordered separately, e.g. as replacement parts, by their catalogue number. The filters are sequentially numbered and come complete with a certificate.

Catalogue Number	Description	Material	Filter Thickn. mm	Position of Peaks at nm	Nominal Absorbance	Absorbance value at nm
666.000	Secondary Standard Set, certified, in storage case consisting of: Holmium Oxide Glass Filter Grey Glass Filters NG 11, NG 5, NG 4 Empty Filter Mount					
666-F0	Empty Filter Mount					
666-F1	Glass Filters, certified	Holmium Oxide Glass	2.1	279, 361, 454, 536, 638		
666-F2	Grey Glass Filters, certified	NG 11	2		0.25	440, 465, 546, 590, 635
666-F3	Grey Glass Filters, certified	NG 5	1.8		0.5	440, 465, 546, 590, 635
666-F4	Grey Glass Filters, certified	NG 4	1.8		1.0	440, 465, 546, 590, 635
666-F7	Didymium Glass Filter, certified	BG 36	1.2	328, 472, 525, 681, 875	between 1.5 and 0.5	280, 300, 320, 340
666.013	Microplate Reader Standard, certified	NG 11, NG 5 NG 4, NG 3		0.25, 0.5, 1.0, 1.5, 2.5		405, 450, 490, 650

available individually

